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Microbial Inoculation Technology of Legume Seed for Crop Improvement

[1]S P Monalisha

Abstract--- *The most well-researched biological nitrogen fixation mechanism is the rhizobia-legume symbiosis. Practically accepted measures to improve the quality of legume body are now the treatment of vegetable seeds with rhizobia. However, due to the low rates of live rhizopia in these products, the effectiveness of certain commercial inoculants cannot be guaranteed in China. A greenhouse experiment was performed to evaluate the effects on alfalfa productivity and nitrogen fixation of various rhizobial inoculant formulations. The use of beneficial microorganisms as alternatives to chemical pesticides and synthetic fertiliser in agricultural production is becoming progressively important. Application of beneficial microorganisms to seeds is an effective method for putting microbial inocula in soil where seedling roots are colonized and protected from insect or soil diseases. The use of beneficial microorganisms as alternatives to chemical pesticides and synthetic fertiliser in agricultural production is becoming progressively important. In view of the long history of Rhizobia spp. inoculation of leguminous plants, there are still very few commercially available microbial seed inoculants and simple laboratory demonstrations of the ability of a wide variety of other beneficial microorganisms to improve crop efficiency. The application of effective rhizobia to the soil and later the rhizosphere of legumes is an efficient and convenient way to inoculate leguminous seed. Nevertheless, its potential is yet to be understood very well. The introduction of high-quality inoculants revolutionized legumes technology in Australia in the 1960s following the widespread collapse in crops.*

Index Terms--- *Alfalfa productivity, Leguminous plants, Nitrogen-fixation mechanism, Rhizobia-legume symbiosis, Rhizobial inoculant formulations, Rhizobia spp., Rhizosphere.*

I. INTRODUCTION

A significant (15-20-fold) increase of the use of synthetic pesticides to control pests, pathogenic and weeds has been necessary in recent decades for the global production of crops, but the growing use of synthetic pesticides is no longer sustainable. Strong consumer pressure led to the elimination of a large number of synthetic pesticides, reduction of maximum residue levels and to regulatory changes promoting more environmentally sound control options. Alfalfa (Medicago sativa L.) is one of China's largest distributed and cultivated forage plants [1]. Alfalfa has a long history of animal production and pasture restoration due to its high nutritional value as a perennial leguminous fodder with exposure to fixed atmospheric nitrogen (N2).

Various studies have been analyzed for legume inoculation technology and further several experimental as well as research studies are going on for the improvement of legume seed inoculation [2]. The researchers in their experiments

Department of Agriculture, Siksha 'O' Anusandhan (Deemed to be University), Bhubaneswar. spmonalisha@soa.ac.in

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demonstrated the N2-fixation criteria with the nodulated legume roots which plays a significant role of the infective agents of nodules. These agents have different roles in their ability in nodulation of different groups of plants [3].

Although, the application of beneficial microorganisms to seeds is not a novel criteria, the inoculation of the legumes have a long history with N2-fixing bacteria and promotes the widespread use of legumes of worldwide significance in food supply. Nonetheless, despite the long history of inoculation of legumes and clearly demonstrated in laboratories, there are still very few commercially available microbial seed inoculants for other beneficial microorganisms. The most researched biological nitrogen fixation system is Rhizobia legume symbiosis [4]. Rhizobia has been shown to increase the amount of nitrogen in host plants by colonizing its roots and exchanging nitrogen fixed by bacteria in the root nodules for plant photosynthesis. Coating alfalfa seeds with inoculated rhizobial products can also increase the size of the seeds, making it uniform in shape and protected against certain pests [5]. This enables mechanized planting and increased fixation of nitrogen and lubricant supply. The effectiveness of coating rhizobic seeds is typically limited because the maintenance of living and working bacterial cells is difficult. Due to the low incidence of live rhizobia in such items and the efficacy of some commercial rhizobic inoculants in China cannot be guaranteed. The survival of rhizobial diseases in the seed coating agent are all influenced by the factors including temperature, humidity and toxic materials. Molybdenum (Mo) plays an essential role in the metabolism of nitrate reductase and nitrogenase [6]. The studies have shown that the molybdenum (Mo) fertilizer have the ability to increase the activities of nitrogenase, glutamine synthase and asparagines synthetase, improvement in the N2 symbiotic root nodule fixation [7] and nitrogenmetabolic activity of plants may increase significantly. Adding a suitable amount of molybdenum to the inoculation formulas could thus increase the seed breathing and enhance rhizobial cell survival. Nevertheless, an overabundance of Mo can change the bacteroid cell membrane permeability to prevent normal ammonia transportation, which helps in inhibiting the function of nitrogenase leading to reduced nitrogen fixation in the root nodules. In addition, the responses to Mo fertilization of various rhizobia strains were suggested to be specific and rely on various factors, in particular the concentration of Mo, in the formulation of the seed-coating. The use of adhesive agents in seed-coats inoculants allows the rhizobial carrier medium to attach to the seeds and prevents or reduces the direct threat of rhizobic substances on the seed surface. According to some of the researchers, it has been reported that the different adsorbing substances can influence rhizobial activity significantly [8]. Because of its low cost and environmental safety, the most common polymerized material used for commercial microorganism inoculation is both carboxymetic cellulose (CMC) and alginate (AER). Such polymers have been shown to protect rhizobia from adverse environmental conditions, even after six months of storage, they maintain a significant number of viable cells. Therefore, it is necessary to evaluate the new formulations for seed coats in order to assess their rhizobial inoculation potential quality and efficacy.

I.I. Inoculation of Legume:

I.I.I. Production and Quality Checking of Legume Inoculants:

Legumes are usually inoculated in rhizobic peat crops. They started to produce finely milled peat in Australia in 1953 as a bacterial carrier. Following wide nodulation failures, the efficiency of inoculants has been improved by

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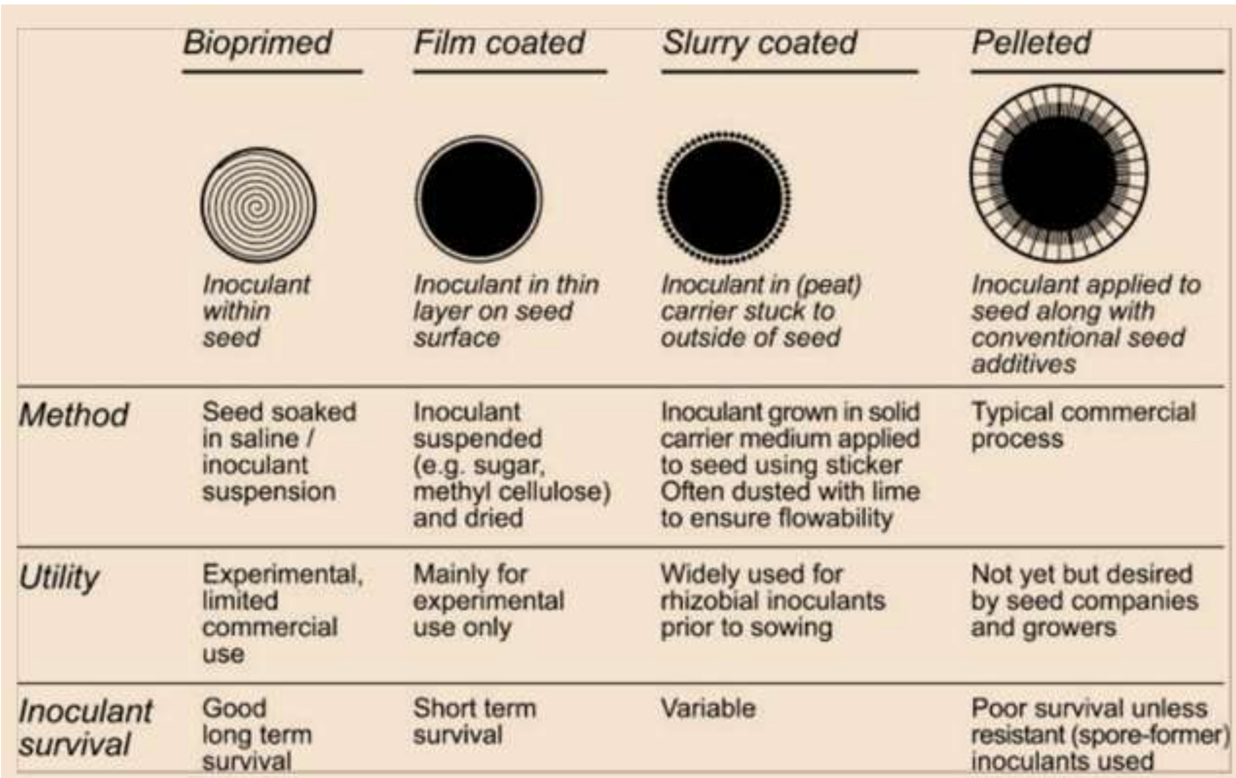
improving five key survival factors in the peat. The first element that has proved significant was the origin of the peat. Rhizobia differed by location and depth of the peat source depending on clover, lucerne and cowpea survival. The peats were evaluated differently based on color and shape, but the authors gave no explanation of the reason for survival differences. Secondly, the pH was shown to be important and the calcium or magnetic carbonate could be used to alter acid peats. Thirdly, the peat sterilization was considered particularly important for slow growing rhizobial growth and survival, preferably through gamma irradiation, which could be the product of faster-growing pollutants. Fourthly, when rhizobia was applied to the previously dried peat at 100 8C, both the heat produced by wetting inoculation and the development of heat treatment inhibitors survived poorly. Eventually, the 40-50% moisture content was suitable for rhizobic strains prepared as peat cultures to grow and thrive. Later salt build-up was found to affect rhizobial survival due to several dry seasons in a peat deposit [9].

Some research work on microbial seed inoculation includes agricultural and seed firms, so that the methods and processes used are scarcely reported and kept as household information or trade secrets as a result of this commercial advantage. Several published research studies have defined methods for the preparation and application of high numbers of microorganisms in research-based seeds. The main methods of seed treatment are shown in the figure 1.



	Bioprimed	Film coated	Slurry coated	Pelleted
	Inoculant within seed	Inoculant in thin layer on seed surface	Inoculant in (peat) carrier stuck to outside of seed	Inoculant applied to seed along with conventional seed additives
Method	Seed soaked in saline / inoculant suspension	Inoculant suspended (e.g. sugar, methyl cellulose) and dried	Inoculant grown in solid carrier medium applied to seed using sticker Often dusted with lime to ensure flowability	Typical commercial process
Utility	Experimental, limited commercial use	Mainly for experimental use only	Widely used for rhizobial inoculants prior to sowing	Not yet but desired by seed companies and growers
Inoculant survival	Good long term survival	Short term survival	Variable	Poor survival unless resistant (spore-former) inoculants used

Fig.1: Properties of methods commonly used for microbial inoculation of seed

Bio-priming is the suspension of seeds in a microbial suspension at a predetermined time and accompanied by the drying of seed, which prevents the onset of germination [10]. Considering the efforts involved in the process, high value crops such as vegetable seeds are best suited for low-medium volume crops. Film coating is used as inoculum suspension or a liquid polymer or adhesive. Popular materials include cellulose methane, paraffin or vegetable oils and

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polysaccharides. In slurry, powdered inoculants or the other carriers (usually peat) are applied with a range of stickers outside of the seeds. However, experimentally, film coating, but slurry covering is widely used in inoculating legume seeds on the farm. Various methods which are used for the research are not suitable for commercial application, and the preference for a pre-inoculated seed that can be bought and used in a manner that is similar to conventionally treated seed is strong for producers and seed companies. Pre-inoculation of seeds poses major scientific, technical and commercial challenges to tackle the rapid growth of the global need for new seed treatments. A variety of analysts have suggested that the biological treatment of seeds has the potential of gaining up to 20% of the world seed treatment market. The fastest-growing seed treatment market in Asia Pacific is expected to grow at a compounded annual rate of 9 percent from 2014 to 2020 with demand for biological seed treatments.

I.II. Inoculation Requirement:

The inoculation decisions generally are based on an established need for experimental plots or a crop or pasture failure cover. The results of a survey on Trifolium subterranean inoculation, grown in New South Wales grounds with nodulation issues, stressed the challenge of predicting the need to inoculate [11]. Of the 30 sites tested, 14 did not respond to inoculation and productive rhizobial strains were found to be naturally occurring. No clear link between the soil types and the existence of successful strains has been observed. In general, lime pellets (super-fine calcareous addition) provided more stable outcomes and superior soils with pH lower than 5.5 to slurry inoculation.

I.III. Techniques of Inoculation:

The introduction of rhizobia in legumes takes place by the inoculation of the seed or soil. The seeds may be inoculated by local seed merchants with coating facilities to be seeded within a week immediately after being sown or custom inoculated within a week [12]. Legumes can also be inoculated and stored on the trade market before they are sold. A pre-inoculated seed is commonly known to this product. In 1972–74 as well as 1999–2002 testing revealed poor survival of the rhizobial inoculum, however, despite a growing demand for pre-inoculated seed since it began in Australia in 1971, the value of the technology had raised a question. Direct soil inoculation with peat inoculants suspended in water or inoculants formulated as liquids or granules shall be used as alternative seed covering methods. Although the techniques of inoculation vary widely, the basic practices are listed in Table 1. Upon inoculating seeds, most of the inoculants are removed when it moves by the machinery, upon dusting with peat mud. The seed is quickly dislodged when the moist inoculum dries out and falls into the seed hopper.

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Table 1: Legume inoculation techniques

Technique	Description
Seed inoculation dusting	Peat inoculants is mixed with the seed without re-wetting
Slurry	Seed is mixed with a water solution of peat often with the addition of an adhesive
Lime or phosphate pelleting	Seed is treated with a slurry peat inoculants flowed by a coating of calcium carbonate (superfine limestone) or rock phosphate
Vacuum impregnation	Rhizobia is introduced into or beneath the seed coat under vacuum
Soil inoculation Liquid inoculation	Peat culture mixed with water or liquid inoculants applied to the seedbed at the time of sowing (liquid inoculants may also be applied to seed)
Granular inoculation	Granules containing inoculums sown with seed in seedbed

However, more peat is retained in the seed coat when peat inoculants are coated with an adhesive. The adhesive should not block the blockages of seed boilers in the coating material, but should prevent the sloughing-off of the coating material. The soybeans were constantly lower than slurry inoculation, after splashing an inoculant on the seed in the planter seed box just before sowing.

I.IV. Challenges in the development of microbial inoculants seed treatment:

Seeds have been treated to increase the profit so that if microbial seed inoculation is not cheap or time consuming, it will not become a viable option, regardless of the effectiveness of the particular inoculant. In order to achieve the full commercial success, farmers should preferably be able to buy seeds inoculated at the same price and use those seeds in the way they have been bought as pesticide-treated seeds. Trade companies will inoculate legumes seeds on request, either after they are sold or before they are sold (pre-inoculation) seed inoculation. Inoculating the seed just before sowing removes the need for an extended inoculant shelf-life. However, seed companies and farmers clearly prefer pre-inoculated seeds, which are prepared weeks and often months before sowing. Therefore, farmers are relieved of the inoculation inconvenience of on-farm seeds and seed companies which supply the pre-inoculated seeds for this product that charge more. Biological seed treatments would preferably have comparable shelf-life qualities to conventionally treated seed, but sustained survival of most seed inoculants is not shown.

I.V. Rhizobial Survival on seed:

I.V.I. Poor survival on seed and its impact on yield:

The species of the Rhizobia which are present on the inoculated seeds takes place very rapidly, specifically at the time of unfavourable environmental conditions. The researchers have recognized the problem of poor rhizobic survival on legumes seed in the early 20th century and its partial improvement by low temperature storage and additives. Inoculation strategies were generally tested in the context of ' grow-out ' test nodulation, and increased nodulation is often related to the better survival. The survival of seed affects the resulting legume yield directly. According to a researcher, the major death factor in the Lupin seed between inoculation and sowing has been identified. Only 4.8% of inoculus remained viable at sowing (3.75 h after inoculation) and 0.83% at 22.5 h in soil, viable at sowing.

I.V.II. Factors affecting rhizobial survival on seed:

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The death of rhizobia is common to all known inoculation procedures and is attributed to three major sources: drying, toxicity and unfavorable storage temperatures of soluble seed coat exudate. One of the most common and major factors responsible for the poor survival on seed is drought. There were two different phases of deaths under the dry conditions which has been reported by an experimental research study of R. leguminosarum bv. Trifolii on glass beads. Following a rapid decrease in cell counts from 0 to 24 hours which resulted in the rapid water loss, a period was followed by a decrease in the water loss and mortality rate. Cells survived best with relative humidity of 100 per cent and, after 27 h, no viable cells were found with relative humidity of less than 60 per cent.

II. CONCLUSION

The currently known inoculation methods restrict the advantages of consistency pulses. Factors that influence the survival of rhizobic plant seed have been identified and improved survival has been observed by research to date when several additives were added. Data from this research was difficult and often speculative to interpret due to the complexity of the surroundings seeds and their disparity in nature. The Mo-enriched rhizobial inoculants increased the alfalfa plant height, dry weight, amount of root nodule, weight of nodules and operation of nitrogenase significantly. The demand is increasing for pre-inoculated seed and a clear indication that an increase in the number of viable rhizobia in seed to the rhizosphere would increase the yield, which means that the rhizobic survival factors in legume seed must be further clarified and the desiccation tolerance of physiological mechanisms understood. In order to assess the resistance to stress factors associated with the drying seedcoats Rhizobium strains may be defined by this criteria.

REFERENCES

[1] C. Fu, T. Hernandez, C. Zhou, and Z. Y. Wang, “Alfalfa (Medicago sativa L.),” in Agrobacterium Protocols: Third Edition, 2014.
[2] J. N. Chianu, E. M. Nkonya, F. S. Mairura, J. N. Chianu, and F. K. Akinnifesi, “Biological nitrogen fixation and socioeconomic factors for legume production in sub-Saharan Africa: A review,” Agronomy for Sustainable Development. 2011.
[3] M. O’Callaghan, “Microbial inoculation of seed for improved crop performance: issues and opportunities,” Applied Microbiology and Biotechnology. 2016.
[4] M. Laranjo, A. Alexandre, and S. Oliveira, “Legume growth-promoting rhizobia: An overview on the Mesorhizobium genus,” Microbiol. Res., 2014.
[5] J. Zhou, B. Deng, Y. Zhang, A. B. Cobb, and Z. Zhang, “Molybdate in rhizobial seed-coat formulations improves the production and nodulation of alfalfa,” PLoS One, 2017.
[6] F. Bittner and R. R. Mendel, “Cell biology of molybdenum,” Plant Cell Monogr., 2010.
[7] S. L. Dwivedi et al., “Advances in Host Plant and Rhizobium Genomics to Enhance Symbiotic Nitrogen Fixation inGrain Legumes,” Adv. Agron., 2015.
[8] A. Broghammer et al., “Legume receptors perceive the rhizobial lipochitin oligosaccharide signal molecules by direct binding,” Proc. Natl. Acad. Sci. U. S. A., 2012.
[9] D. Lesueur, R. Deaker, L. Herrmann, L. Bräu, and J. Jansa, “The production and potential of biofertilizers to improve crop,” in Bioformulations: For Sustainable Agriculture, 2016.
[10] A. Mahmood and R. Kataoka, “Potential of biopriming in enhancing crop productivity and stress tolerance,” in Advances in Seed Priming, 2018.
[11] E. A. Drew and R. A. Ballard, “Improving N2 fixation from the plant down: Compatibility of Trifolium subterraneum L. cultivars with soil rhizobia can influence symbiotic performance,” Plant Soil, 2010.
[12] A. K. Simonsen, R. DInnage, L. G. Barrett, S. M. Prober, and P. H. Thrall, “Symbiosis limits establishment of legumes outside their native range at a global scale,” Nat. Commun., 2017.

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